PORCINE COLLAGEN MATRIX WITH AND WITHOUT ENAMEL MATRIX DERIVATIVE FOR THE TREATMENT OF GINGIVAL RECESSION DEFECTS

BY

CHRISTOPHER T. BARTH, DMD
LIEUTENANT, DENTAL CORPS
UNITED STATES NAVY

A thesis submitted to the Faculty of the Periodontics Graduate Program
Naval Postgraduate Dental School
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Master of Science
In Oral Biology

June 2016

Naval Postgraduate Dental School
Uniformed Services University of the Health Sciences
Bethesda, MD

CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

Christopher T. Barth

has been approved by the Examining Committee for the thesis requirement for the Master of Science degree in Oral Biology at the June 2016 graduation.

Thesis Committee:

Glen Imamura, DDS, MS
CAPT (Ret), DC, USN, Professor, Dental Research
Thesis Supervisor

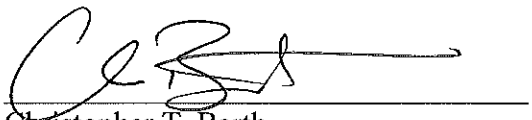
Ivan Roman, DDS, MS
CAPT, DC, USN
Chairperson Periodontics Department

Matthew B. Miller, DDS, MS
CDR, DC, USN
Program Director Periodontics Department

The author hereby certifies that the use of any copyrighted material in the thesis manuscript titled:

PORCINE COLLAGEN MATRIX WITH AND WITHOUT ENAMEL MATRIX DERIVATIVE FOR THE TREATMENT OF GINGIVAL RECESSION DEFECTS

is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.



Christopher T. Barth
Periodontics Department
Naval Postgraduate Dental School
May 2016

ABSTRACT

PORCINE COLLAGEN MATRIX WITH AND WITHOUT ENAMEL MATRIX DERIVATIVE FOR THE TREATMENT OF GINGIVAL RECESSION DEFECTS

CHRISTOPHER T. BARTH, DMD
PERIODONTICS 2016

Thesis directed by: Glen Imamura, DDS, MS
CAPT (Ret), DC, USN
Professor, Dental Research
Naval Postgraduate Dental School

Matthew B. Miller, DDS, MS
CDR, DC, USN
Program Director, Periodontics
Naval Postgraduate Dental School

Introduction

The purpose of this study is to compare the effectiveness of a porcine collagen matrix (CM), with or without the addition of enamel matrix derivative (EMD) in the treatment of Miller Class I, II or III recession defects. This will be a prospective, single-blinded, randomized, split-mouth study. One defect will receive CM+EMD (test), while the other will receive CM (control) alone.

Methods

The treatment of 30 similarly sized Miller class I, II or III recession defects on single-rooted teeth using coronally advanced flaps with CM + EMD or CM alone will be evaluated. The subjects will be in good health, non-smokers, periodontally healthy except for recession, have good oral hygiene and have no contraindications to periodontal surgery. Subjects will have matching recession defects that measure within 1 mm of each other on single-rooted

teeth. One defect will be randomly assigned as the test group receiving CM + EMD, and the other as the control group receiving only CM. Measurements will be made using a UNC-15 periodontal probe, custom acrylic stent and digital calipers and will include: probing depth (PD), clinical attachment level (CAL), vertical recession (RD) and width of keratinized tissue (KTW). The measurements will be taken at baseline (on the day of surgery), and at 3 and 6 months post-surgery.

Results

Currently this research protocol has been prepared to be submitted to the IRB.

Discussion

Research will commence following IRB approval.

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

CTG - Connective Tissue Graft

CM - Collagen Matrix

EMD - Enamel Matrix Derivative

CEJ - Cemento-Enamel Junction

MGJ - Mucogingival Junction

AG - Attached Gingiva

KG - Keratinized Gingiva

FGM - Free Gingival Margin

PD - Probing Depth

CAL - Clinical Attachment Level

CAF - Coronally Advanced Flap

EDTA - Ethyldiaminetetracetic Acid

S-CEJ - Stent to CEJ

S-BOP - Stent to Base Of Pocket

S-FGM - Stent to FGM

S-MGJ - Stent to MGJ

SNOSE - Sequentially Numbered Opaque Sealed Envelope

BID - Twice per day

qXh - Every X hours where X is an integer

mg - Milligram

TBSP - Tablespoon, PO Take by mouth

CHAPTER I: INTRODUCTION AND LITERATURE REVIEW

A tooth consists of a crown, which is composed of an inner core of dentin and outer shell of enamel and root, composed of dentin covered by a thin layer of cementum. The crown is demarcated from the root at the cemento-enamel junction (CEJ) where the enamel meets the cementum. Gingiva describes the keratinized epithelium that surrounds teeth and is continuous with the periodontal ligament within the gingival sulcus and the mucosa of the oral cavity at the mucogingival junction (MGJ). The immobile component of gingiva is bound to the bone and is called attached gingiva (AG), whereas the mobile component surrounding the tooth is not directly attached to the bone and is termed free gingiva (Figure 1). Both free and attached gingiva make up keratinized gingiva (KG). The most coronal portion of the gingiva that ends on the tooth is defined as the free gingival margin (FGM).

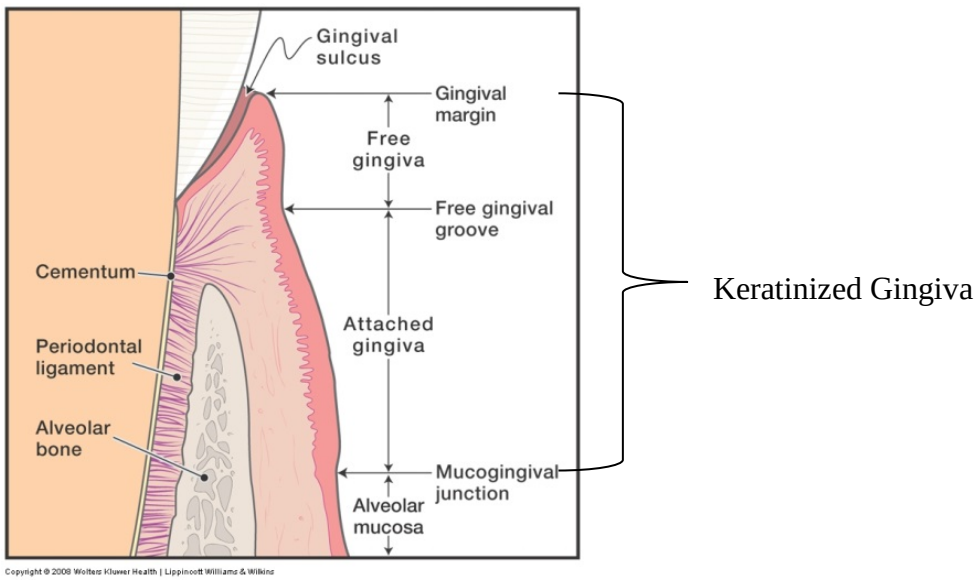


Figure 1

According to the American Academy of Periodontology, recession is defined as the location of the gingival margin apical to the cemento-enamel junction (CEJ).[1] The occurrence of recession is a relatively common finding, affecting 61.3 million adults over the age of thirty with greater than or equal to one millimeter of recession. Recession has been shown to increase in severity and extent with age. Those 30 to 39 years of age have a prevalence of 37.8% on an average of 8.6% of teeth, while 90.4% of 80 to 90 year-old subjects were found to have recession on 56.3% of teeth. Recession is more prevalent in males than females and more common among African Americans than Caucasians.[2] Causes of recession have been attributed to anatomic factors, periodontal disease, tooth malposition, chronic trauma from abrasive or incorrect tooth brushing technique and pathologic insult to the periodontium caused by bacterial plaque. Studies have reported injuries to the gingiva from chemicals or use of smokeless tobacco can also cause recession.[2,3]

In 1985, P.D. Miller presented a classification system for recession (Figure 2). His system was designed to predict the success of coverage achieved with surgery based on the level of the interproximal bone, the presence of KG and the position of the tooth within the alveolus. According to his classification, a recession defect is considered Class I if there is no interproximal bone loss, the tooth is in normal position and the defect does not extend to the MGJ. A Class II defect carries the same criteria except the recession extends to or beyond the MGJ. These two classifications are associated with a predictability of 100% root coverage when surgically corrected. The two remaining classifications, Class III and IV, involve interproximal bone loss and/or malposition of

the teeth in the arch and are associated with less than 100% root coverage or no anticipated gain respectively.[4]

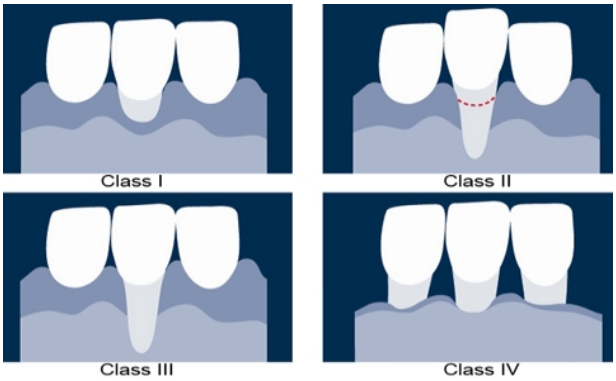


Figure 2

Not every case of recession requires correction. However, there are specific indications for surgical intervention such as patient aesthetic concerns, which can be important to a person's identity and self-image.[5] Another indication is root sensitivity which often results in pain to cold, heat and even touch leading to an impaired ability to eat or drink and brush one's teeth. Studies have shown soft tissue coverage procedures can lead to a decrease in sensitivity of the associated tooth.[6-8] Root caries is a concern because the dentinal surfaces are exposed to the oral environment. Unlike enamel, dentin is less mineralized and consists of tubules that extend from the pulp to the outer surface of the tooth root. If left exposed, these channels are susceptible to bacterial invasion. Surgical coverage of the root surface has been shown to be effective in reducing the incidence of decay through a connective tissue or epithelial attachment to the tooth.[9]

The width of KG is also an important factor in the maintenance of gingival health and its absence is an indication for correction of recession. Lang, in 1972, suggested a minimum of 2 mm of keratinized tissue, 1 mm of which is attached, is needed around

teeth. He noted the presence of chronic inflammation and progression of recession when there was less than 2 mm of KG.[8-11] In 1985, Kennedy and Dorfman demonstrated that a group of untreated, non-compliant control subjects with minimal KG had significantly increased plaque and inflammation compared to a group with adequate KG at baseline.[11] From these historical studies, both observational and prospective in nature, it is evident that the level or amount of KG is crucial to the periodontal health of the patient.[11,12]

In 2016, Chambrone and Tatakis demonstrated that patients with existing recession defects are at a significantly increased risk for further progression of recession. They noted 78.1% of patients that started with recession, experienced increased depth of recession; concluding that untreated defects have a high probability of becoming worse in the long term.[13]

Mucogingival Therapy: Overview

The correction of recession defects usually requires a surgical procedure. There is a plethora of available techniques to treat gingival recession. These can include repositioning the existing gingival tissue with techniques such as a lateral pedicle flap, coronally advanced flap, double papilla flap or semi-lunar repositioned flap. These techniques aim to reposition the neighboring tissue to cover the recession defect. Also, there are different grafting techniques that can be employed to transplant tissue from one site to another. These include connective tissue autograft, allograft or xenograft.[14-16] Finally, grafts and pedicle flaps can be used in conjunction with each other in any possible combination. In addition, numerous biologic agents aimed at augmenting healing are available such as platelet derived growth factor, bone morphogenic protein-2

and enamel matrix derivative.[17] Of these various surgical techniques available to treat gingival recession, the sub-epithelial connective tissue graft (CTG) or simply CTG is considered the gold standard.[6,18]

Mucogingival Therapy: The Connective Tissue Graft

The CTG has the ability to change the morphology of the tissue surrounding the tooth from non-keratinized mucosa to keratinized attached tissue. In a series of studies on monkeys, Karring (1971) demonstrated that when a connective tissue graft from a keratinized source such as the palate heals at the transplanted site, the new epithelium that grows over the grafted tissue will be keratinized tissue even if it was previously non-keratinized.[19] This is why the source for connective tissue autografts is from a location with keratinized tissue such as the patient's palate, edentulous ridges or maxillary tuberosity. Also, the CTG has the ability to create a new connective tissue attachment onto the previously diseased root surface.[20]

Numerous studies have shown that the use of CTG with various pedicle flaps such as lateral pedicle flap, coronally positioned flap or double papilla flap, effectively decreases the amount of recession, increases the level of attachment, decreases probing depth and increases the amount of keratinized tissue.[14-16] In a study by Bittencourt et al. in 2009, an average of 96.83% of root coverage was achieved in Miller Class I defects using CTG and coronally advanced flap (CAF). These results stayed consistent from 6 to 30 months post-operatively. Also, Harris (2002) showed mean root coverage of 97% at 13 weeks in Miller class I or II defects using a variety of pedicle flaps. Root coverage improved to an average of 98% at 27.5 months.[18] Not only does this show that CTG

results are stable long term, but that they actually may improve over time. Systematic reviews and meta-analyses have also demonstrated the superiority of CTG compared to other techniques such as guided tissue regeneration or free gingival grafting.[14,21]

One of the main drawbacks to the CTG is the need for a second surgical site from which to harvest. This adds to the risk of surgical complications such as infection, nerve damage, bleeding and pain and also increases the time of surgery. In 2006, Griffin showed that by eliminating the need for a second surgical site, the probability of postoperative moderate to severe bleeding and swelling decreased by 24%. Powell, in 2005, demonstrated a 3.66% infection rate in CTG compared to overall infection rates for periodontal surgical procedures of 2.09%. Also, the amount of tissue available for grafting is limited by patient anatomy affecting the number of teeth that can be addressed in one surgery. If there are more teeth to be grafted than available graft, additional surgical procedures may be needed.[22,23]

The endpoint of success for surgical treatment of recession is coverage of exposed root through coronal reposition of the free gingival margin (FGM). Ideally, there will be an increase in KG, shallower PD, decreased CAL and increased tissue thickness which would lead to a more stable tissue attachment to the tooth.

Mucogingival Therapy: Xenogenic Collagen Membrane

In an effort to define an alternative and equally successful graft modality that overcomes the second site “problem”, xenogenic collagen membranes (CM) can be used in the correction of gingival recession or augmentation of KG. The structure of the membrane is usually collagen derived from an equine, porcine or bovine source. These

membranes offer a unique advantage as alternatives to CTG because they eliminate a second surgical site and have relatively unlimited availability.

In 2010, Osteohealth introduced Mucograft®, a bi-layered membrane composed of Type I and III collagen. It is derived from veterinary certified pig tendon, sterilized by gamma irradiation and has a low antigenicity in conjunction with good biocompatibility. The material is approximately 2.5-5.0 mm thick and is cleared by the FDA for root coverage, increasing keratinized tissue, covering of implants placed immediately and alveolar ridge reconstruction.[24] A compact outer layer contributes to protection, structure and allows for better control during suturing; while the inner layer is thick, porous and spongy, providing a suitable environment for early vascularization and promotion of cellular recruitment.[25] Mucograft® is safe for use in humans and is routinely used currently in periodontal practice for the correction of recession defects.[26-28]

Collagen matrices used for gingival recession coverage result in similar outcomes to those achieved with connective tissue autografts.[26-28] In 2009, Sanz et al. conducted a randomized retrospective clinical trial consisting of 20 patients followed for 1, 3 and 6 months with regard to keratinized tissue gained through CTG vs CM augmentation. They found a statistically significant amount of keratinized tissue achieved with both grafting materials (2.6 mm and 2.5 mm respectively) and a lower patient morbidity associated with the collagen matrix.[28] Similarly, in one of the first clinical studies looking at recession coverage with CM compared to CTG, McGuire et al. (2010), found mean CAL, PD and KG width to be statistically significant compared to baseline. All parameters tested for differences between treatment groups also showed equivalence and at 6 months, no difference could be made in regards to color or texture.[16] These studies

demonstrate there is support for clinical results using collagen matrices that are at best, equivalent to the standard CTG. To date, there have been no reports of infections or any inflammatory reactions resulting from the use of this xenograft. It is safe, predictable and the risk to the patient is exceptionally low.[16,28]

Mucogingival Therapy: Enamel Matrix Derivative

Of the biological factors available to treat gingival recession defects, one of the most widely used is enamel matrix derivative (EMD). EMD is a derivative of embryonal porcine enamel and is composed primarily of amelogenins as well as other proteins such as tufelin, ameloblastin and amelin. The molecular composition of EMD has been shown to affect gene expression, protein production and differentiation of various cell types crucial to the formation of periodontal ligament and osteoblastic cell types.[29] EMD has also been shown to enhance angiogenesis and stimulate endothelial cells, which favors early healing of the soft tissue.[30] EMD is FDA approved for application to root surfaces to treat recession defects.[31]

Ample research exists demonstrating the efficacy and safety of EMD leading to increases in attachment level, root coverage and reduction in probing depths.[32-34] McGuire and Nunn demonstrated an average root coverage of 94% in EMD+CAF when compared to CTG+CAF which attained 96.3% mean root coverage. Complete root coverage was attained in 78% of all cases. In a ten year follow up study, these results remained stable with the exception of PD reduction and width of KG.[35] In 2010, Henriques et al. showed increased attachment levels and decreased probing depths using EMD with a CTG compared to CTG alone.[33] Additionally in 2011, Rasperini et al.

demonstrated improved reduction of recession depth in test group (EMD+CAF+CTG) compared to control (CAF+CTG).[36] In a meta-analysis completed by Cheng et al. in 2015, it was concluded that EMD can improve probing pocket reduction and increased keratinized tissue width.[37]

EMD is used with a root surface modifier known as ethylenediaminetetraacetic acid (24% EDTA). As per the manufacturer's recommendations and instructions for use, EDTA is applied to the tooth surface for 2 minutes prior to the application of EMD. The rationale for its use is to remove debris from the root surface and expose collagen fibrils to allow fibroblast to directly contact the dentinal collagen. Studies that have utilized EDTA report no altered healing or serious side effects associated with its use.[31, 32]

Study Rationale

Currently, there is evidence demonstrating comparable improvements in correcting gingival recession using CM or CTG.[16, 35] Also, EMD has demonstrated enhanced clinical outcomes when used with CTG or alone.[37] Therefore, the question raised is if the use of EMD with CM can enhance the clinical outcomes related to root coverage procedures compared to using CM alone. To date, there are no published studies that have explored this approach. The purpose of this study is to determine if the addition of enamel matrix derivative results in increased root coverage compared to CM alone in patients with Miller Class I, II or III recession defects undergoing a coronally advanced flap.

CHAPTER II: MATERIALS AND METHODS

Thirty subjects will be consented and enrolled to obtain 24 evaluable subjects (estimated attrition: 20%) with matching Miller Class I, II or III recession defects (Please see Appendix A for flow diagram of study). Corrective surgical treatment will be offered to these patients in accordance with accepted indications for root coverage procedures. A study investigator will initiate the consent process described in this protocol.

Clinical Sequence (with introduction of study and consent):

1. Patient is referred for a periodontal evaluation following any indicated non-surgical therapy.
2. Any patient who has bilateral mucogingival defects with Miller Classification I, II or III will be asked by their provider if they are interested in participating in a study about mucogingival defect corrective therapy.
3. If the patient expresses interest, the provider will give the patient a one page overview that summarizes the protocol.
4. If the patient does not wish to participate, he or she will be offered any indicated periodontal treatment in accordance with his/her needs and the rules and regulations of the department.
5. If the patient wishes to participate in the study, the provider will ask a study investigator to meet with the patient to discuss the study and consent process.
6. If the patient does not consent to be in the study, therapy will continue as described in step 4.
7. If the patient consents, the following process ensues.

Following Consent:

1. Dental impressions:
    a. Maxillary and mandibular impressions will be made using an irreversible hydrocolloid material (alginate) in stock impression trays. The impressions will be poured with dental stone for models, also called casts. Specific recession defects will then be identified.
2. Laboratory acrylic stent:
    a. A customized acrylic stent for making measurements at the recession sites will be fabricated.
    b. Moldable light curable acrylic material will be adapted to the cast in proximity to the site of the recession defect.
    c. The stent will direct the probe to facilitate precise, reproducible measurements.
    d. A fissure bur will be used to cut a “tube” in the stent. The tube will accommodate a periodontal probe which will allow a reproducible, fixed reference point for repeat measurements intra-orally.
    e. Measurements using the acrylic stent will be collected at baseline, 3 and 6 months post surgically.
    f. Clinical data will be collected by blinded associate investigators. Clinical measurements will be made at baseline, 3 months and 6 months and recorded on data collection sheet (Appendix D). The data collection sheets will be stored in a locked cabinet located in the PI’s office at NPDS.

g. Clinical measurements will be taken using a UNC 15 probe and a custom stent to allow standardized placement of the probe. The *top* of the stent will serve as the standardized point of reference. The probe will be inserted through a port in the stent with a rubber stopper demarcating the distance from the probe tip to the top of the stent. (Appendix F) This distance will be measured using digital calipers manufactured by CMT Industrial capable of measuring to 0.01 mm.

h. The following measurements will be made to the nearest 0.01 mm using digital calipers:

    i. Stent-Cemento-enamel junction (S-CEJ)

    ii. Stent-Base of pocket (S-BOP)

    iii. Stent-Free Gingival Margin (S-FGM)

    iv. Stent-Mucogingival junction (S-MGJ)

i. Study variables will be mathematically determined using the following formulas:

    i. Probing depth (PD): S-BOP minus S-FGM

    ii. Clinical Attachment Level (CAL): S-BOP minus S-CEJ

    iii. Recession(REC): S-FGM minus S-CEJ

    iv. Keratinized tissue width(KG): S-MJG minus S-FGM

<u>Randomization and De-Identification procedure:</u>

3. Confounding variables that could influence the outcomes of these procedures include the surgical technique, subject's ability to heal, anatomy, defect size and tissue morphology. Therefore, Miller Class I, II or III defects will be paired and matched

within the same subject. This allows the patient-to serve as her own control, mitigating differences between subjects if there was a separate control population. Both defects will be surgically corrected on the same day. Before surgery, a sequentially-numbered, opaque, sealed envelope (SNOSE) will be opened by the surgeon that will direct which defect (left or right) will receive which treatment condition (test or control), and in which order the surgeries should be performed (test first or control first). SNOSE will be pre-generated; separate randomization groups will be stratified subsequent to enrollment for Miller's Class I, II, and Class III defects.

4. Each subject will be given an identifying number based on the chronology of their enrollment. The PI will be the only person to have access to the subject code key. The PI will be the only one to have access to the subject's study record. Also, the PI or AI will enroll subjects. Therefore, investigators who make measurements at each surgical site will be blinded to which tooth received CM only and which tooth received CM + EMD.

Surgical Procedure:

1. Females of child bearing age will be asked to complete a HCG urinalysis prior to the surgical procedure. If the results of the HCG test are positive, the subject will be exited from the study.
2. Prior to surgical procedure, in line with standard protocol at the Periodontics Department, participants will be offered the option of having the surgery

performed using either oral anxiolysis or intravenous moderate sedation. The use of sedation will not affect the surgical procedure.

3. The surgical provider will be either a board certified staff periodontist or a 2nd or 3rd year periodontal resident. All surgical providers will be briefed in the protocol. All surgeries will encompass the steps listed below:
    a. Baseline measurements using the stent will be collected at this time by an investigator.
    b. Administration of oral anxiolysis or IV moderate sedation if patient desired and indicated.
    c. Patient will be anesthetized using local anesthetic.
    d. SNOSE will be opened identifying the test and control sites and the order of the procedures.
    e. The exposed portion of the root will be prepared and cleaned using a combination of hand instruments and ultrasonic instruments.
    f. A recipient pouch at the treatment site will be created via an intra-sulcular incision with two vertical incisions and combination full/partial thickness flap. Passive coronal positioning of the flap at or slightly above the level of the CEJ will be achieved.
    g. The facial portion of the adjacent papillae to the treated tooth will be de-epithelialized to create a bleeding CT bed to which the flap will be sutured after graft placement.
    h. The exposed root surface will be conditioned using 24% EDTA (per the manufacturer's recommendations) for 2 minutes, then irrigated copiously

with sterile saline.

i. Both test and control sites will receive Mucograft® (cut to a size 6 mm larger than the defect both laterally and apically). The Mucograft® will be secured using a sling suture with resorbable suture material around the tooth.

j. Test sites will receive 1 mL of Emdogain® that will be applied to the Mucograft® once it is secured around the tooth. Control sites will not receive this Emdogain® application.

k. The flap will be coronally advanced to cover the graft and then secured to the de-epithelialized papilla at or coronal to the level of the CEJ using a non-resorbable suture.

Post-Operative Care

Verbal and written post-operative instructions (See Appendix C). The subjects will be asked to avoid any brushing or flossing of the grafted sites for 4 weeks following the surgery to avoid compromising the graft by physical trauma. Subjects will also be asked to not engage in any strenuous activity immediately following the procedure.

a. All participants will receive the following post-operative medication regimen unless otherwise medically contraindicated:

- 0.12% Chlorhexidine, 1 bottle: Rinse and spit BID with 1 TBSP as directed on the bottle as a replacement for brushing around the surgical site (to reduce plaque).
- Pain medication consisting of any of the following regimens (alone or in combination)

1. Ibuprofen 800 mg, Take 1 tab PO q6-8h for moderate pain
2. Hydrocodone/Acetaminophen 5/325 mg, Take 1-2 tab PO q6h prn severe/breakthrough pain
3. Acetaminophen 325 mg: Take 1-2 tabs PO q4h for moderate pain
4. Oxycodone 5mg: Take 1 tab PO q4h prn severe/breakthrough pain.

b. Subjects will be recalled at 1, 2 and 4 weeks to monitor post-operative healing and remove plaque/deposits at the surgical site. At week 2 subjects will be instructed to swab the surgical site with a cotton tip applicator dipped in chlorhexidine in lieu of whole mouth rinsing. At 4 weeks, they will be instructed to resume normal hygiene using a soft toothbrush.

c. Subjects will be recalled at the, 3 and 6 month mark (each +/- 2 weeks) following the surgical procedure to assess healing, remove plaque, and reinforce oral hygiene. Three and 6 month measurements will be collected at these appointments by an AI or PI. At 6 months, the subject will be referred back to his/her primary provider for continuation of dental care.

Data Analysis Plan:

A patient flow diagram as outlined in the CONSORT statement will be presented to describe enrollment, intervention allocation, follow-up, and analysis. Significance for all independent analyses will be set at a global alpha = 0.05. A table of baseline patient demographic and clinical characteristics will be presented using means with standard deviations, medians with interquartile ranges, or minimum-maximum ranges and counts with

percentages.

To address the study objectives:

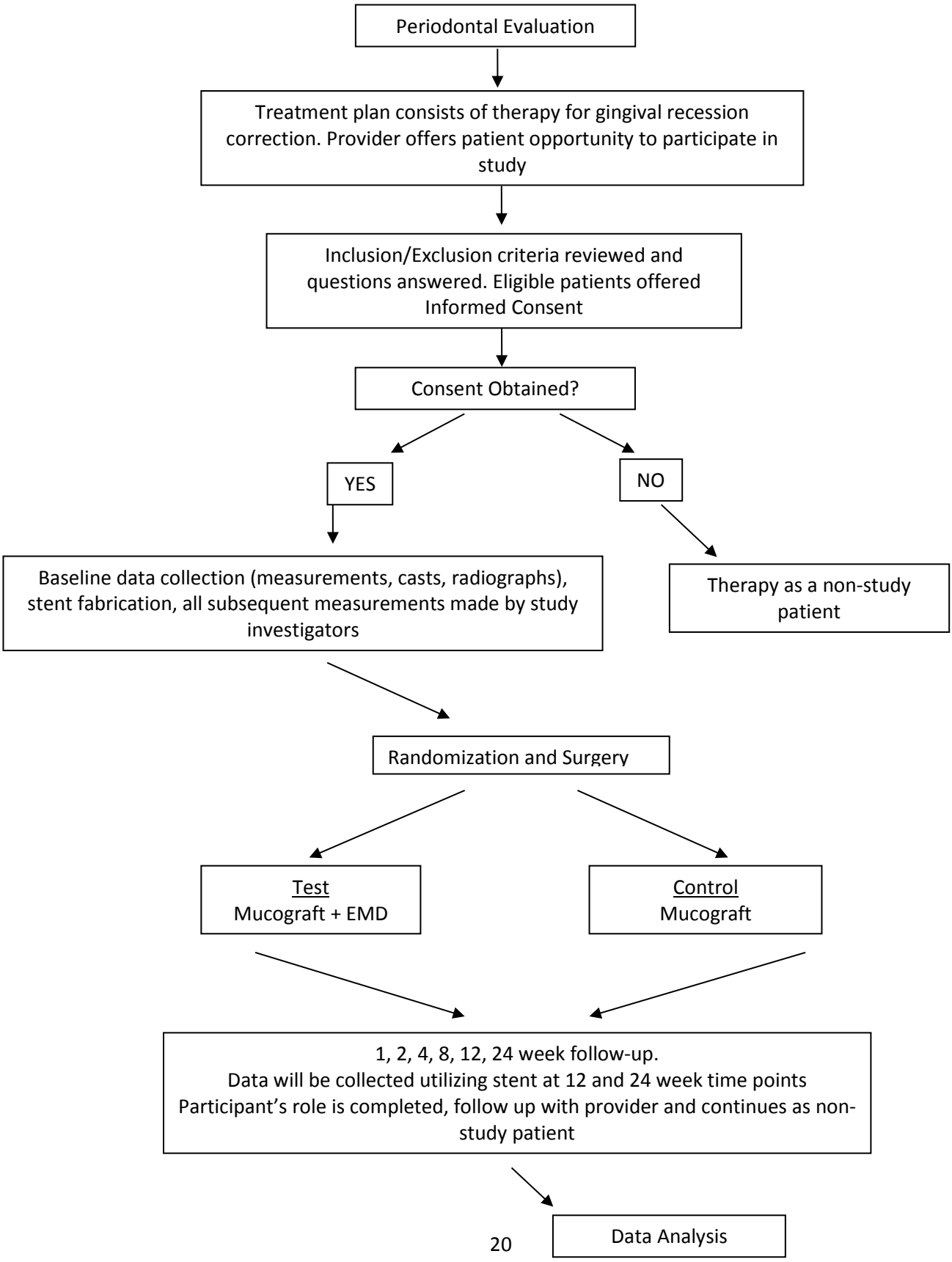
1. The CM + EMD and the CM alone will be evaluated for correction of recession depth. The primary dependent measure will be the size (mm) of recession depth. To evaluate the difference between these treatments over time, a linear mixed-effects model or generalized estimating equation will be used. The Wilcoxon Signed Rank Test (paired t-test) will be used for paired (split-mouth) inter-treatment (CM+EMD and CM alone) comparisons at each of the measurement times (baseline, 3 months, and 6 months) and to compare, across the treatments, the change in recession depth from each time point with the baseline (i.e., 3 months- baseline, 6 months – baseline). Additionally, to evaluate the intra-treatment efficacy, the Wilcoxon Signed Rank Test (paired t-test) will be used to evaluate significant changes in recession depth across time (comparing each to baseline: 3 months, and 6 months). Other clinical variables, including the clinical attachment level, probing depth, and keratinized tissue width will be similarly evaluated. Additionally, we will similarly compare changes in percent coverage, which can be derived using the size of the existing defect and other physiological parameters of the tooth. For this last measure, we will also evaluate, using a logistic regression, if CM + EMD results in a higher likelihood of promoting 100% root coverage than CM alone.
2. We will evaluate the inter-rater reliability between the set of human measurements obtained using the stent & digital caliper. Here, we will calculate Cronbach's Alpha and the associated ICC values.

3. Covariates in each of the above analyses, such as recession defect class, surgery order or laterality, patient age, measuring associate investigator, and performing surgeon may be included.

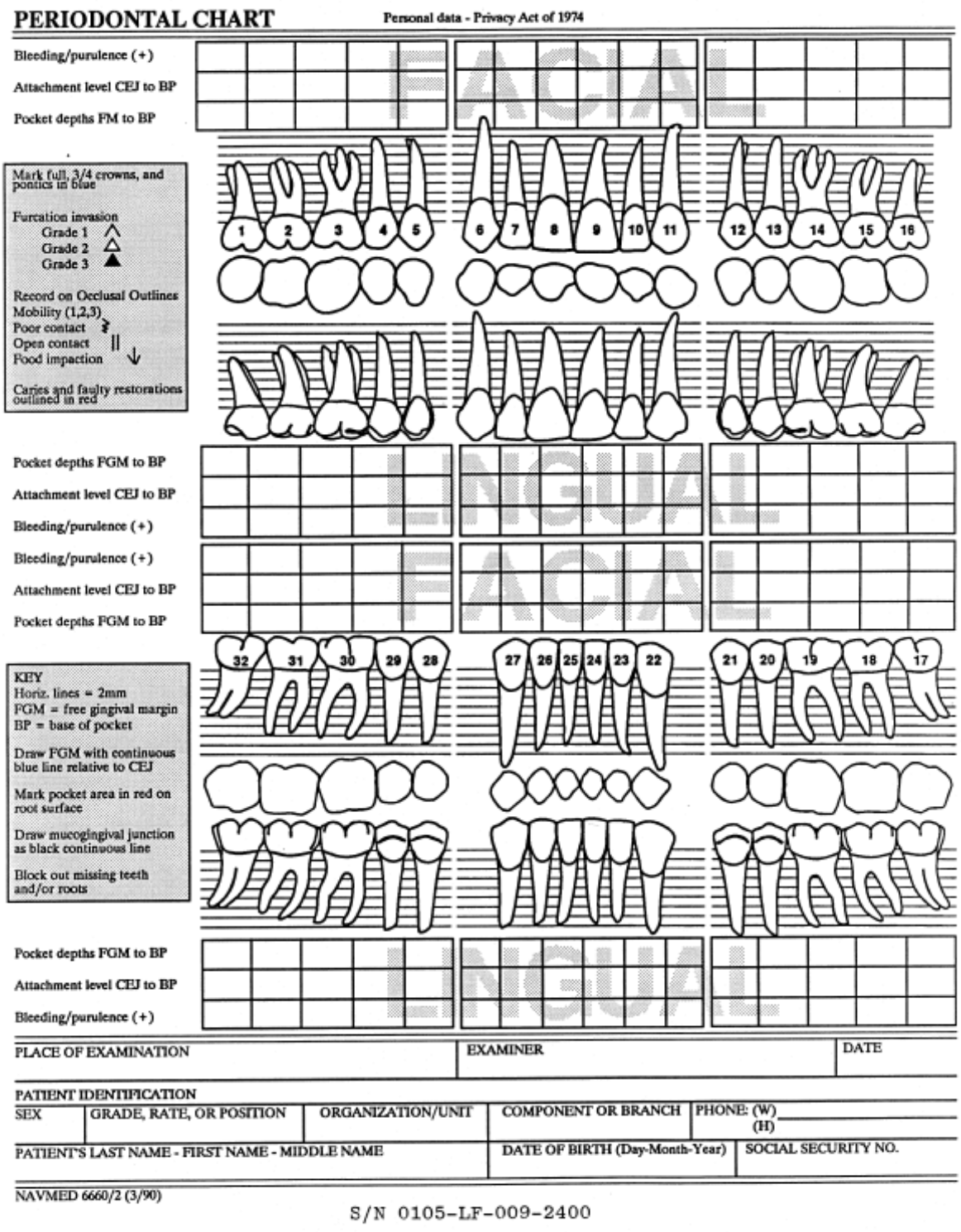
CHAPTER III: CONCLUSIONS

Research will commence following IRB approval. The standards for both clinical parameters and patient satisfaction have been set high with the connective tissue autograft technique. Recently, several products have come to light that give the CTG a “run for its money” demonstrating comparable and in some cases, equivocal outcomes. The emphasis must be placed on equivocal or comparable, not superior, because we have yet to discover a technique that outperforms the CTG. In the periodontal literature, the success of these techniques is well established but there is no definitive evidence demonstrating how they could be enhanced. The goal we are striving to achieve in this study is to do just that, with the combination of two excellent materials used commonly in practice today.

APPENDIX A: FLOW DIAGRAM OF STUDY DESIGN



APPENDIX B: COMPREHENSIVE PERIODONTAL CHARTING FORM



APPENDIX C: EXAMPLE OF NPDS PERIODONTICS DEPARTMENT POST-OPERATIVE INSTRUCTIONS

PERIODONTICS DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Bethesda, Maryland

For best healing and a minimum of complications, please read and follow these instructions carefully

You may have been given one or more of these medications:

PAIN MEDICATIONS:	_____	Motrin 800 mg:	*1 tablet every 8 hours.* Do not double up on dosage.
	_____	Norco 5/325 mg:	*1 tablet every 6 hours for pain control.* It can be taken in addition to ibuprofen. This medicine can make you drowsy. Therefore, do not drive or operate machinery while taking this drug. Additionally, do not take with alcoholic beverages; the alcohol will make you sleepier, but will not decrease your comfort.
ANTIBIOTICS:	_____	Doxycycline 100 mg:	*2 tablets the day of surgery, then 1 tablet every day for 30 days.*
	_____	Amoxicillin 500 mg:	*1 tablet four times a day for 7 to 10 days.*
	_____	Clindamycin 300 mg	*1 tablet four times a day for 7 to 10 days.*
RINSES:	_____	Peridex (Perioguard	*1 bottle, rinse twice-a-day as directed on the bottle, starting the day following surgery.* Do not brush or floss at the surgical site unless instructed to do so.
ANTI-INFLAMMATION:	_____	Medrol Dose Pack:	Take as directed on the package, starting today. Be sure and take the full first row of tablets (first six tablets) today.

The following are a list of post-operative considerations during healing:

BLEEDING:	There may be slight bleeding from the surgical for 1-2 days after surgery. Your saliva may appear slightly reddish. This is common. If you notice an increase in bleeding please contact us.
SUTURES/STITCHES:	You may have sutures placed in your mouth. They may have to be removed in the future. Please leave the sutures alone as much as possible. Early removal or the loss of sutures may impair healing.
DRESSINGS:	There may be a gummy type of dressing or pack over the surgical area. It is there for your comfort. If it falls out before your first post-operative appointment and you are comfortable, it is fine to leave it out. If the surgical site is uncomfortable and you would like the dressing replaced please contact me.
DIET:	It is very important to maintain a soft diet for at least a week. Chew as much as possible on the side opposite the surgery. This is not the time to start a diet. Please maintain your caloric and fluid intake as at pre-surgical levels. You will not heal well if you are dehydrated or undernourished. Please do not drink using a straw.
ORAL HYGIENE:	It is very important not to brush or floss the surgical site until given express instructions. Normal brushing and flossing procedures can traumatize the tissue and impair healing. You may brush and floss those areas not affected by the surgery. To keep bacteria under control a prescription mouth rinse has been written for you. Initially, use the mouthwash as a rinse. Later you may be instructed to use a cotton-tipped applicator, dipped in the mouthwash, to swab along the gum line of the surgery site. Use a capful (15ml) of the mouthwash twice a day, morning and bedtime, after brushing/flossing your non-surgically treated teeth. You may notice a mild tooth staining as a result of the mouthwash. This is not permanent; the stain will be removed with scaling/polishing at your follow-up appointments. Please do not use a Water-Pik or other irrigator unless instructed to do so.
PHYSICAL ACTIVITY:	Avoid strenuous physical activity (to include running and heavy lifting) for 72 hours. Additionally, no vigorous spitting, rinsing, or speaking (yelling). Forceful movements at the site of surgery will negatively affect healing.
SWELLING:	You may experience some swelling. This is common and usually peaks at 2-3 days after surgery. Thereafter you should expect to see a return to normal. To decrease swelling you can apply ice to the site for the first 3-4 hours after surgery. Please call if the swelling appears to increase after the third day, or if you are concerned.
SMOKING	Smoking is deleterious to healing. We advise you to stop smoking for as long as possible after surgery. Stopping smoking will improve potential healing and also improve your overall periodontal health.
FOR SINUS LIFT SURGE PROCEDURES	You may also have received nasal decongestant tablets and spray. Please use these medications as directed on the package. In addition, avoid blowing your nose. If you need to sneeze, please sneeze with your mouth open. Please inform your doctor if you develop sinus congestion that is not minimized with your medications or if you notice any bleeding or discharge from your nose.

If you have any problems or questions, please do not hesitate to call me at 301-295-0077. If there is an emergency you may page your doctor through an automated system. Instructions will be given after dialing 1-800-759-8888. The PIN# for your doctor is _____

Your follow up appointment is scheduled for: _____ _____

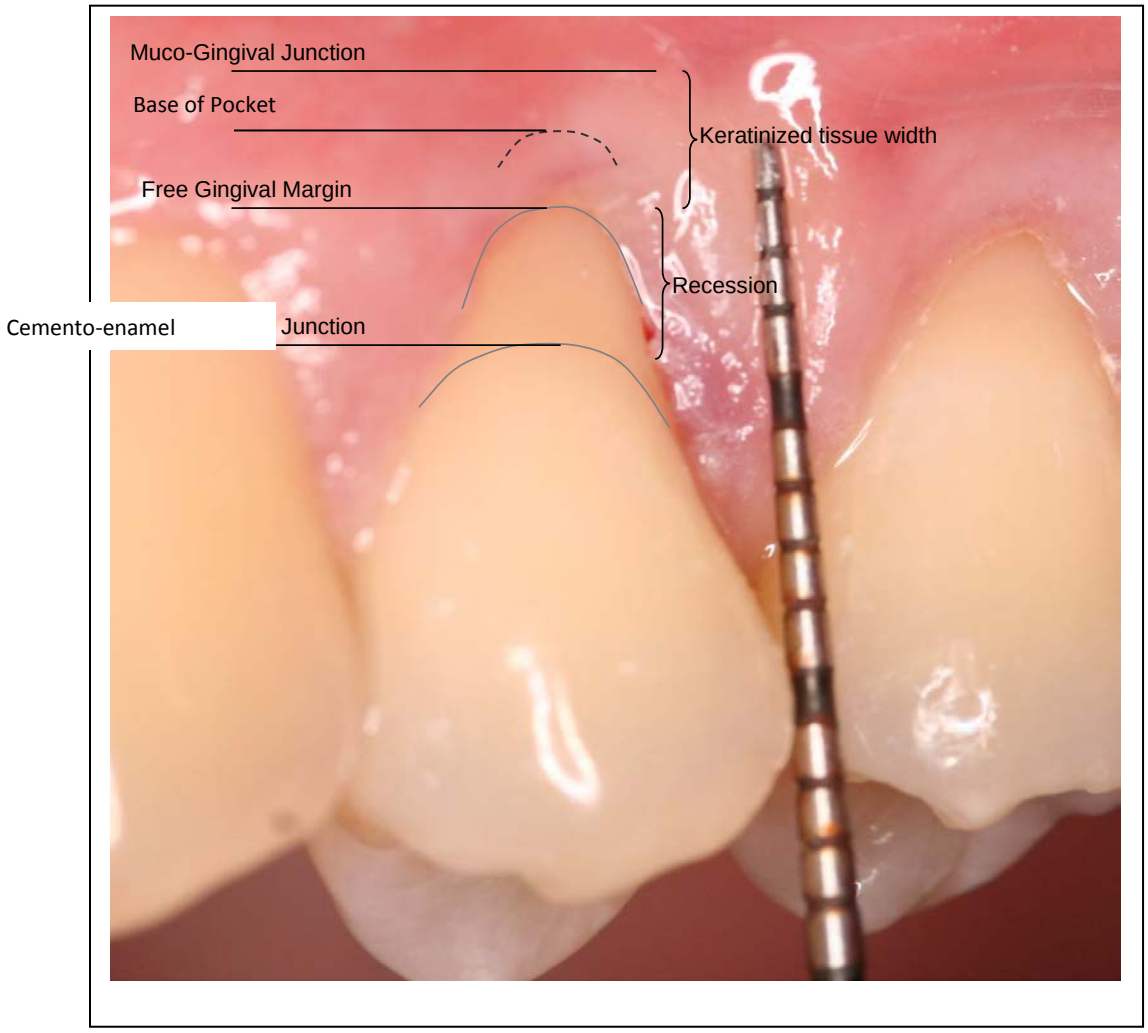
NPDS Periodontics

APPENDIX D: EXAMPLE OF DATA COLLECTION SHEET

Subject ID	
Date	
Provider	
Experimental side (R or L)	

Measurements (to nearest 0.01 mm)	**Tooth#**	**Tooth#**
Stent-CEJ		
Stent-BOP		
Stent-FGM		
Stent-MGJ		

APPENDIX E: MEASUREMENT LANDMARKS OF STUDY



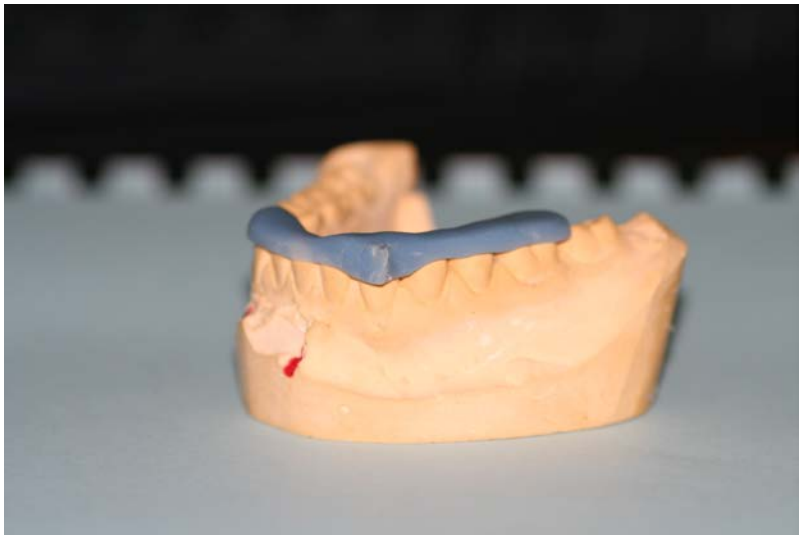
Amount of Recession = Cemento-enamel Junction to Free Gingival Margin

Probing depth = Free Gingival Margin to Base of Pocket

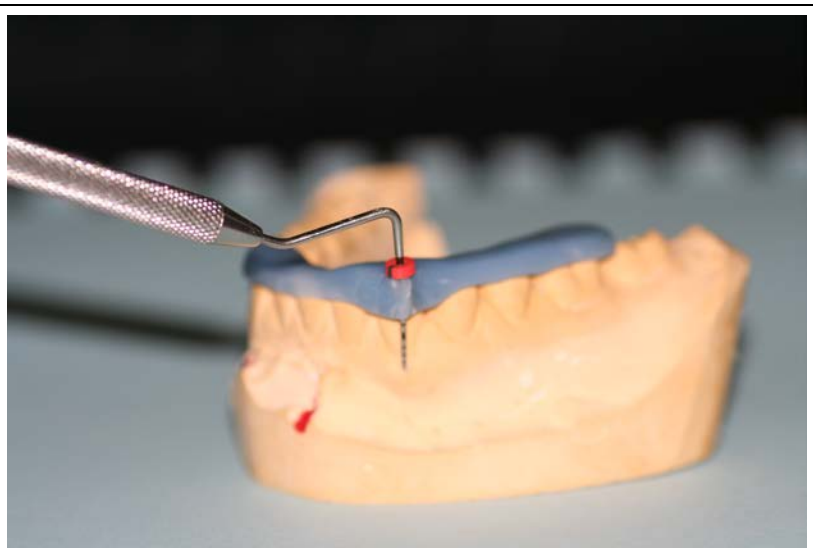
Clinical Attachment Level = Cemento-enamel Junction to Base of Pocket

Width of Keratinized Tissue = Free Gingival Margin to Mucogingival Junction

APPENDIX F: EXAMPLE OF MEASUREMENT STENT



The measurement stent will be fabricated from a stone model of the patient's mouth.



The stent will direct the probe to facilitate precise, reproducible measurements.

REFERENCES

1. American Academy of Periodontology, Glossary of Terms: Task Force Update 2015.
2. Albandar JM, Kingman A. Gingival recession, gingival bleeding, and dental calculus in adults 20 years of age and older in the United States, 1988-1994. J Periodontol 1999; 70:30-43.
3. Robertson PB, Walsh M, Green J, Ernster V, Grady D, Hauck W. Periodontol effects associated with the use of smokeless tobacco. J Periodontol 1990; 61:438-443.
4. Miller PD. A classification of marginal tissue recession. Int J Periodontics Restorative Dent 1985; 5:8-13.
5. Kokich VO, Kokich VG, Kiyak HA. Perceptions of dental professionals and laypersons to altered dental esthetics: Asymmetric and Symmetric situations. *Am J Orthod Dentofacial Orthop* 2006;130:141-151
6. Oates TW, Robinson M, Gunsolley JC. Surgical therapies for the treatment of gingival recession. a systematic review. Ann Periodontol 2003; 8:303-320.
7. Bouchard P, Etienne D, Ouhayoun J-P, Nilveus R. Subepithelial connective tissue grafts in the treatment of gingival recessions. A comparative study of 2 procedures. J Periodontol 1994; 65:929-936.
8. Pini-Prato G, Tinti C, Vincenzi G, Magnani C, Cortellini P, Clauser C. Guided tissue regerenation versus mucogingival surgery in the treatment of human buccal gingival recession. J Periodontol 1992; 63:919-928.

9. Goldstein M, Nasatzky E, Goultschin J, Boyan B, Schwartz Z. Coverage of carious roots by a subepithelial connective tissue graft. Am J Dent 2002;15:143-148.
10. Lang N, Loe H. The relationship between the width of keratinzed gingiva and gingival health. J Periodontol 1972;43:623-627
11. Kennedy JE, Bird WC, Palcanis KG, Dorfman HS. A longitudinal evaluation of varying widths of attached gingiva. J Clin Perio 1985; 12: 667-675.
12. Dorfman HS, Kennedy JE, Bird WC. Longitudinal evaluation of free autogenous gingival grafts: a four year report. J Periodontol 1982; 53: 349-352.
13. Chambrone L, Tatakis DN. Long-term outcomes of untreated buccal gingival recession. A systematic review and meta-analysis. J Periodontol 2016; 15: 1-17.
14. Chambrone L, Pannuti CM, Tu YK, Chambrone LA. Evidence-based periodontal plastic surgery. II. An individual data meta-analysis for evaluating factors in achieving complete root coverage. J Periodontol 2012; 83:477-490.
15. Harris R. Root Coverage with Connective Tissue Grafts: An Evaluation of Short- and Long-Term Results. J Periodontol 2002;77:1054-1059.
16. McGuire MK, Scheyer ET. Xenogenic collagen matrix with coronally advanced flap compared to connective tissue with coronally advanced flap for the treatment of dehiscence- type recession defects. J Periodontol 2010; 81:1108-1117.
17. Ramseier CA, Resperini G, Batia S, Giannobile WV. Advanced reconstructive technologies for periodontal tissue repair. Perio 2000; 59:185-202.
18. Bittencourt S, Ribeiro E, Sallum EA, Sallum AW, Nociti Jr. FH, Casati MZ. Semilunar coronally positioned flap or subepithelial connective tissue graft for the

treatment of gingival recession: a 30-month follow-up study. J Periodontol 2009; 80: 1076-1082.

19. Karring T, Lang NP, Loe H. The role of gingival connective tissue in determining epithelial differentiation. J Periodontal Res; 10: 1-11.
20. Bruno JF, Bowers GM. Histology of a human biopsy section following the placement of a sub-epithelial connective tissue graft. Int J Periodontics and Restorative Dent 2000; 20: 225-231.
21. Chambrone L, Skekava F, Araujo MG, Pustiglioni FE, Chambrone LA, Lima LA. Root-coverage procedures for the treatment of localized recession-type defects: a Cochrane systematic review. J Periodontol 2010; 81:452-478.
22. Griffin TJ, Cheung WS, Zavras AI, Damoulis PD. Postoperative complications following gingival augmentation procedures. J Periodontol 2006; 77:2070-2079.
23. Powell CA, Mealey BL, Deas DE, McDonell HT, Moritz AJ. Post-surgical infections: prevalence associated with various periodontal surgical procedures. J Periodontol 2005; 76:329-333.
24. U.S. Food and Drug Administration, Office of Device Evaluation, 510(k) Summary. Mucograft® collagen matrix K073711/approval letter, May 30, 2008. Retrieved September 15, 2013, from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K073711
25. Schwarz F, Rothamel D, Herten M, Sager M, Becker J. Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. Clin. Oral Impl. Res. 17, 2006; 403–409.

26. Cardaropoli D, Tamagnone L, Roffredo A, Gaveglio L. Treatment of gingival recession defects using coronally advanced flap with porcine collagen matrix compared to coronally advanced flap with connective tissue graft: A randomized controlled clinical trial. J Perio 2011; 11: 2-15.
27. Jepsen K, Jepsen S, Zucchelli G, Stefanini M, de Sanctis M, Baldini N, Greven B, Heinz B, Wennstrom J, Cassel B, Vignoletti F, Sanz M. Treatment of gingival recession defects with a coronally advanced flap and a xenogenic collagen matrix: a multicenter randomized clinical trial. J Clin Perio 2013;40:82-89.
28. Sanz M, Lorenzo R, Aranda JJ, Martin C, Orsini M. Clinical evaluation of a new collagen matrix Mucograft® prototype) to enhance the width of keratinized tissue in patients with fixed prosthetic restorations: A randomized clinical trial. J Clin Perio 2009;36:868–876.
29. Grandin HM, Gemperli AC, Dard M. Enamel matrix derivative: A review of cellular effects in vitro and a model of molecular arrangement and functioning. Tissue Engineering Part B: 2012; 18:181-202.
30. Thoma DS, Villar CC, Carnes DL, Dard M, Chun Y, Cochran DL. Angiogenic activity of enamel matrix derivative (EMD) and EMD-derived proteins: an experimental study in mice. J Clin Perio 2011; 38: 253-260.
31. U.S. Food and Drug Administration, Office of Device Evaluation, Premarket Approval of Biora AB Emdogain® PMA P930021/pre-market approval, November, 21, 1996. Retrieved September 15, 2013, from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P930021

32. Alkan EA, Parlar A. Enamel matrix derivative (Emdogain®) or subepithelial connective tissue graft for the treatment of adjacent multiple gingival recessions: a pilot study. Int J Periodontics Restorative Dent 2013; 33:619-625.
33. Henriques PS, Pelegrine AA, Nogueira AA, Borghi MM. Application of subepithelial connective tissue graft with or without enamel matrix derivative for root coverage: a split-mouth randomized study. J Oral Sci 2010; 52:463-471.
34. Koop R, Merheb J, Quirynen M. Periodontal regeneration with enamel matrix derivative in reconstructive periodontal therapy: a systematic review. J Periodontol 2012; 83;707-720.
35. McGuire MK, Scheyer TE, Nunn M. Evaluation of human recession defects treated with coronally advanced flaps and either enamel matrix derivative or connective tissue: comparison of clinical parameters at 10 years. J Periodontol 2012; 83:1353-1362.
36. Rasperini G, Roccuzzo M, Francetti L, Acunzo R, Consonni D, Silvestri M, Subepithelial connective tissue graft for treatment of gingival recessions with and without enamel matrix derivative: a multicenter, randomized controlled clinical trial. Int J Periodontics Restorative Dent 2011; 31:133-139.
37. Cheng Gl, Fu E, Tu YK, Shen EC, Chiu HC, Yuh DY, Chiang CY. Root coverage by coronally advanced flap with connective tissue graft and/or enamel matrix derivative: a meta-analysis.